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NEW HAPALINDOLES FROM THE CYANOPHYTE *HAPALOSIPHON LAINGII*

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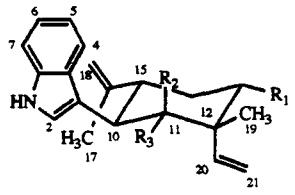
ABSTRACT.—Seven indole alkaloids have been isolated from the cultured cyanophyte *Hapalosiphon laingii*. In addition to the known metabolites 12-*epi*-hapalindole E isonitrile [**1**], 12-*epi*-hapalindole C isonitrile [**2**], hapalindolinone A [**3**], and hapalindolinone B [**4**], *H. laingii* contains three new hapalindoles, 12-*epi*-hapalindole H [**5**], 12-*epi*-hapalindole G [**6**], and 12-*epi*-hapalindole Q isonitrile [**7**], the structures of which were elucidated by interpretation of their spectral data.

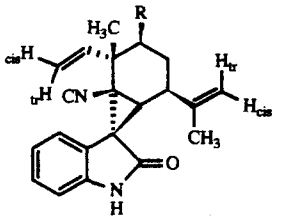
The hapalindoles are a class of alkaloids of mixed biogenesis, presumably derived from tryptophan and a monoterpene unit, found in some blue-green algae of the Stigonematales. These alkaloids are responsible, at least in part, for the fungicidal activity of the lipophilic extract of several species of *Hapalosiphon* (1–6) and *Fischerella* (4, 7–9) as well as of *Westiellopsis prolifica* (4) and *Westiella intricata* (5). To date, about fifty different hapalindoles and hapalindole-type alkaloids have been isolated and identified, most of them possessing an isonitrile or isothiocyanate function.

Recently, we have initiated a screening program to evaluate laboratory-cultivated blue-green algae as a source of interesting new bioactive molecules. The extracts of about 50 blue-green algal strains from marine or terrestrial habitats were tested for their toxicity against fish (*Lebistes reticulatus*), *Artemia* nauplii and/or fungi (*Aspergillus niger, Fusarium oxysporum*, and *Alternaria tenuis*). Several extracts were found to be active. In particular, the lipophilic extract of a novel Stigonematales species, described as *Hapalosiphon laingii* L. Hoffmann (10) and isolated from the surface of dead coral debris lying on the ground of a forested coral island in Papua New Guinea, exhibited toxic activities (10). The alga was mass cultivated and the lipophilic extracts subjected to successive gel filtration, Si gel flash chromatography, and reversed-phase hplc. Seven hapalindole alkaloids were isolated from the active fractions. Four are already known compounds which were identified on the basis of their spectral properties as 12-*epi*-hapalindole E isonitrile [**1**] (5,8), 12-*epi*-hapalindole C isonitrile [**2**] (5,8), hapalindolinone A [**3**] (7), and hapalindolinone B [**4**] (7), respectively. We describe here the elucidation of the novel structures of 12-*epi*-hapalindole H [**5**], 12-*epi*-hapalindole G [**6**], and 12-*epi*-hapalindole Q isonitrile [**7**].

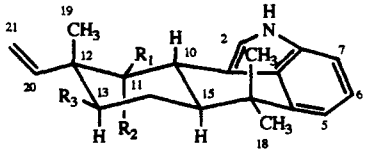
The hreims of compound **5** displayed a molecular ion at *m/z* 304.1943 that established the elemental composition $C_{21}H_{24}N_2$ (calcd 304.1939, Δ −0.4 mDa). The compound exhibited a typical indole uv spectrum [λ max 221 (ϵ) (28140), 280 (5150), 291 (4130)] as well as ir and ^{13}C-nmr peaks [ν max 2134 cm^{-1}; δ_C 157.5] characteristic of an isonitrile function. The ^{1}H-nmr signals of **5** are presented in Table 1 and the ^{13}C-nmr signals in the Experimental. The assignments of both ^{13}C- and ^{1}H-nmr spectra were based upon analyses of the 1D (broad-band proton decoupling and NOED) and 2D (HMQC and COSY) nmr spectra. Comparison of these data with those of hapalindole H



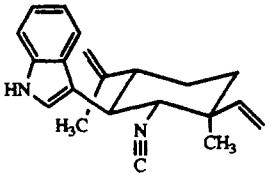
	R_1	R_2	R_3
1	Cl	-NC	H
2	H	-NC	H
10	H	H	-NCS



3 R=Cl
4 R=H



	R_1	R_2	R_3
5	-NC	H	H
9	H	-NC	Cl



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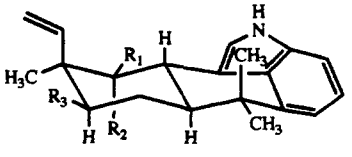

	R_1	R_2	R_3
6	H	-NC	Cl
8	-NC	H	H

[8] (2) clearly indicated that compound **5** is the C-12 epimer of **8**. Indeed, the only significant differences were variations of chemical shifts restricted to proton and carbon atoms surrounding C-12. Moreover, as with hapalindole H, the vicinal coupling constants measured in the ^{1}H-nmr spectrum of **5** indicated that the protons on C-10, C-11, and C-15 were axial ($J_{10,11}=J_{10,15}=11$ Hz). In the nOe difference spectra, irradiation of the Me-18 singlet (δ 1.46) induced an appreciable increase in the H-5 signal, and irradiation of the Me-17 singlet (δ 1.15) caused an nOe in the H-10 signal. These observations indicated that both compounds have the same relative configuration at the stereogenic atoms C-10, C-11, and C-15. However, contrary to what was observed for hapalindole H, decoupling experiments performed with compound **5** showed a strong positive nOe in the H-21 trans and H-10 signals when the Me-19 singlet (δ 1.32) was irradiated, whereas irradiation of the H-20 signal (δ 5.89) indicated a close proximity between this proton and H-11. All these results indicated that Me-19 was axial and the geminal vinyl group equatorial and that the preferred conformation of the vinyl group is one in which the C-20,C-21-double bond is s-cis with respect to Me-19. For all these reasons, compound **5** is 12-*epi*-hapalindole H and its relative configuration is 10*S**,11*S**,12*R**,15*S**.

Hreims established the molecular formula of **6** as $C_{21}H_{23}N_2Cl$ (calcd 338.1550; observed 338.1559; Δ −0.9 mDa). The uv [λ max (ε) 222 (37220), 280 (8200), 291 (6640)] and ir [ν_{NH} 3674 cm^{-1} and ν_{NC} 2141 cm^{-1}] spectra supported the presence of an indole chro-

mophore and an isonitrile group. The ^{1}H-nmr spectral data of **6** are presented in Table 1. It showed the same number of proton signals as that of hapalindole G [**9**] (2). The most significant differences between the two compounds were variations of the chemical shifts for H-11, H-13, H-20, and Me-19 suggesting that **6** and **9** are epimeric at C-12. Careful analysis of the coupling constants of all the protons of the 6-membered ring led to axial and equatorial assignments identical to those of **9**. These results were further supported by difference nOe spectroscopy experiments. Irradiation of the Me-18 signal (δ 1.52) induced strong positive nOes in the H-5, H-15, and H-14eq signals, whereas irradiation of the Me-17 (δ 1.14) or of the H-20 (δ 6.32) signals caused significant increases in the H-10 and H-14ax signals. This indicated that the vinyl group, the isonitrile function and H-13 are axially disposed. In addition, irradiation of the Me-19 signal (δ 1.54) indicated close proximity between this methyl group and H-11 and H-13. This irradiation also caused a strong positive nOe in the H-21 trans signal suggesting that, as for 12-*epi*-hapalindole H, the C-20,C-21-double bond is s-cis with respect to Me-19. Compound **6** is therefore 12-*epi*-hapalindole G and its relative configuration is 10*S**,11*R**, 12*S**,13*R**,15*S**.

Compound **7** exhibited a uv spectrum [λ max (ε) 222 (24160), 283 (4850), 291 (4300)] typical of an indole chromophore and ir and ^{13}C-nmr peaks [ν_{NC} 2140 cm^{-1}; δ_C 156.7] characteristic of an isonitrile. A molecular formula of $C_{21}H_{24}N_2$ could be deduced for the molecular ion (calcd *m/z* 304.1939; observed 304.1948; Δ −0.9 mDa) by hreims. The ^{1}H-nmr spectrum of **7** taken at room temperature in $CDCl_3$ presented a small number of broadened peaks between 2.5 and 4 ppm that became sharp at higher temperature (75°) indicating that **7** exists in at least two slowly exchanging conformations. As a result, all the ^{1}H-

TABLE 1. ^{1}H-Nmr Data for **5–7**.[a]

Position	Compound		
	5	**6**	**7**[b]
1	8.02 br s	8.05 br s	8.01 br s
2	7.59 dd (2,2)	6.93 dd (1.5,1.5)	7.02 d (2.5)
4	—	—	7.65 br d (8)
5	7.03 m	7.04 m	7.16 ddd (1,7,8)
6	7.18 m	7.20 m	7.08 ddd (1,7,8)
7	7.18 m	7.20 m	7.33 br d (8)
10	3.23 dd (11,11)	3.31 br d (12)	3.11 dd (11,11)
11	3.54 d (11)	4.40 d (3)	3.76 br d (11)
13ax	1.45 ddd (3,11,14)	4.30 dd (4,12)	1.65 to 1.84 4H
13eq	1.78 ddd (3,3,14)	—	
14ax	1.59 dddd (11,11,13,13)	1.99 ddd (12,12,12)	
14eq	1.85 dddd (3,3,3,13)	2.37 ddd (3,4,12)	
15	1.49 ddd (3,11,11)	2.09 ddd (3,12,12)	2.68 br dd (11,11)
17	1.15 s	1.14 s	1.53 s
18	1.46 s	1.52 s	4.53/4.47 br s
19	1.32 s	1.54 s	1.32 s
20	5.89 dd (11,17)	6.32 dd (11,18)	5.91 dd (11,17)
21 trans[c]	5.20 d (17)	5.37 d (18)	5.16 br d (17)
21 cis[c]	5.17 d (11)	5.42 d (11)	5.11 br d (11)

[a]Measured in $CDCl_3$.
[b]$C_2D_2Cl_4$ at 75°.
[c]Trans and cis with respect to H-20.

nmr spectra of **7** were recorded at 75° in $C_2D_2Cl_4$. Comparison of the nmr data (Table 1) with those of hapalindole Q [**10**] (2) indicated that **7** is 12-*epi*-hapalindole Q isonitrile. Consistent with this proposal were the coupling constants of H-10, H-11, and H-15 which indicated an axial orientation for these protons. This was further supported by difference nOe experiments. Irradiation of the signal for H-15 showed an appreciable nOe in the H-11 signal but none in the H-10 signal. Moreover, a strong nOe was observed in the H-10 signal when Me-19 was irradiated, thus proving that the methyl group on C-12 is also axial. Compound **7** thus has the relative configuration 10*R**,11*R**,12*S**,15*R**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were taken on a Philips PU 8700 uv-vis spectrophotometer in MeOH. Ir spectra were recorded on a Bruker IFS 25 instrument as a film on a NaCl disk. Eims measurements were performed on a VG Micromass 7070F and hreims on a Fisons VG Autospec. The ^{1}H- and ^{13}C-nmr spectra were recorded in $CDCl_3$ at 600 and 150.87 MHz (Varian Unity 600 instrument) or at 250 and 62.86 MHz (Bruker WM 250 instrument). The chemical shifts are reported in ppm (δ) and the coupling constants in Hz. Optical rotations were measured on a Perkin-Elmer 141 polarimeter at 589 nm (Na D line) in a 1-dm cell. Flash liquid chromatography was performed over Macherey-Nagel Si gel (0.04–0.063 mm), and tlc analysis on Polygram SilG/UV_{254} precoated plates (0.25 mm). Hplc were performed using a Waters 510 instrument and a L-4000 Merck-Hitachi uv variable detector (λ set at 220 nm).

CULTURE CONDITIONS.—*Hapalosiphon laingii* L. Hoffmann (Stigonematales, Cyanophyceae), designated strain 89-785/4, was isolated from the surface of dead coral debris lying on the ground at Laing Island, a forested coral island on the northern coast of Papua New Guinea. Clonal cultures were prepared by repeated subculture on solidified media. The alga was cultured in 2-liter glass bottles containing $BG11_0$ medium (12), viz., in g/liter for K_2HPO_4.3 H_2O, 0.04; $MgSO_4$.7 H_2O, 0.075; $CaCl_2$.2 H_2O, 0.036; citric acid, 0.006; ferric citrate, 0.006; EDTA, 0.001; Na_2CO_3, 0.02; trace metal solution, 1 ml/liter. The trace metal solution consisted of, in g/liter, H_3BO_3, 2.86; $MnCl_2$.4 H_2O, 1.81; $ZnSO_4$.7 H_2O, 0.222; Na_2MoO_4.2 H_2O, 0.39; $CuSO_4$.5 H_2O, 0.079; $Co(NO_3)_2$.6 H_2O, 0.0494. Cultures were mixed by continuous air-bubbling and they were maintained under a continuous illumination at an incident intensity of 100 μeinstein.m^{-2}.s^{-1} from cool-white fluorescent tubes and at a temperature of 22°. After 4 to 5 weeks the alga was harvested by filtration.

EXTRACTION AND ISOLATION.—The freeze-dried alga (5.25 g) was triturated with CH_2Cl_2, EtOH, and MeOH to yield 198, 536, and 273 mg extracts, respectively. The CH_2Cl_2 and EtOH extracts which showed activity in the *Artemia* nauplii toxicity assay (11) were combined, partitioned with a mixture [*i*-PrOH-EtOAc-hexane-H_2O, 2:4:3:6] and the organic layer evaporated to dryness. The residue (424 mg) inhibited at 0.5 mg per disk the growth (>4 mm) of *Aspergillus niger*, *Fusarium oxysporum*, and *Alternaria tenuis* in soft agar disk-diffusion assays (12) and killed guppies (*Lebistes reticulatus*) after 2 to 3 h of exposure at 50 mg/liter. The LD_{50} value for *Artemia* nauplii was 20 mg/liter. The residue was then applied to a column of Sephadex LH-20 and eluted with EtOH. Two out of the five fractions collected were toxic toward *Artemia*: fractions 3 (104 mg) and 5 (59 mg). Both fractions were then flash chromatographed over a column of Si gel eluting with a solvent gradient system from 100% hexane to 100% EtOAc. Fractions with the same tlc profile were combined to yield individual fractions which were further purified by hplc over a Merck Lichrospher 100 RP-18 column (10 μm; CH_3CN-H_2O, 7:3) to give 12-*epi*-hapalindole E isonitrile [**1**] (14.8 mg), 12-*epi*-hapalindole C isonitrile [**2**] (4.6 mg), hapalindolinone A [**3**] (14.2 mg), hapalindolinone B [**4**] (1.2 mg), 12-*epi*-hapalindole H [**5**] (6 mg), 12-*epi*-hapalindole G [**6**] (0.6 mg), and 12-*epi*-hapalindole Q isonitrile [**7**] (6.6 mg).

12-epi-*Hapalindole E isonitrile* [**1**].—Mp 169–172.5°; $[\alpha]^{25}D$ +57.5° (*c*=1.2, CH_2Cl_2); uv (MeOH) λ max (ε) 221 (28400), 282 (5000), 290 sh (4480) nm; ir ν_{NH} 3424 cm^{-1} and ν_{NC} 2142 cm^{-1}; eims *m/z* 338/340 (80:27, M^+), 182 (89), 168 (100), 130 (95); ^{1}H- and ^{13}C-nmr spectra were identical to those reported by Schwartz *et al.* (8); COSY, HMQC, and NOED spectra fully supported the identification.

12-epi-*Hapalindole C isonitrile* [**2**].—$[\alpha]^{25}D$ +17.9° (*c*=0.35, CH_2Cl_2); uv (MeOH) λ max (ε) 222 (26900), 283 (4460), 290 sh (4060) nm; ir ν_{NH} 3396 cm^{-1} and ν_{NC} 2141 cm^{-1}; eims *m/z* 304 (100, M^+), 168 (80), 130 (95); the ^{1}H-nmr spectrum was identical to that reported by Stratmann *et al.* (5); COSY and NOED spectra fully supported the identification.

Hapalindolinone A [**3**].—$[\alpha]^{25}D$ −35.2° (*c*=0.35, CH_2Cl_2); uv (MeOH) λ max (ε) 223 (24380), 261 (4260) nm; ir ν_{NH} 3174 cm^{-1}, ν_{NC} 2123 cm^{-1}, ν_{CO} 1705 cm^{-1}; eims *m/z* 352 (54, M^+),

317 (100), 237 (64), 212 (80), 210 (76), 184 (84), 180 (97); ^{1}H- and ^{13}C-nmr spectra were identical to those reported by Schwartz *et al.* (7).

Hapalindolinone B [**4**].—$[\alpha]^{25}D$ −82.5° (*c*=0.1, CH_2Cl_2); uv (MeOH) λ max (ε) 223 (24600), 261 (4570) nm; ir ν_{NH} 3208 cm^{-1}, ν_{NC} 2124 cm^{-1}, ν_{CO} 1703 cm^{-1}; eims *m/z* 318 (74, M^+), 250 (61), 212 (100), 210 (78), 184 (93), 146 (83), 132 (79); ^{1}H- and ^{13}C-nmr spectra were identical to those reported by Schwartz *et al.* (7); COSY and NOED spectra fully supported the identification.

12-epi-*Hapalindole H* [**5**].—Mp 187–189°; $[\alpha]^{25}D$ +217.3° (*c*=0.163, CH_2Cl_2); uv (MeOH) λ max (ε) 221 (28140), 282 (5150), 291 sh (4130) nm; ir ν_{NH} 3409 cm^{-1} and ν_{NC} 2134 cm^{-1}; hreims *m/z* 304.1943 (calcd for $C_{21}H_{24}N_2$, 304.1939); eims 304 (100, M^+), 289 (49); ^{1}H-nmr data, see Table 1; ^{13}C nmr δ 118.4 (C-2), 113.4 (C-3), 140.7 (C-4), 112.8 (C-5), 122.9 (C-6), 108.2 (C-7), 133.5 (C-8), 125.1 (C-9), 35.9 (C-10), 65.9 (C-11), 37.4 (C-12), 35.9 (C-13) 20.6 (C-14), 49.7 (C-15), 40.5 (C-16), 24.7 (C-17), 24.9 (C-18), 17.0 (C-19), 145.3 (C-20), 113.6 (C-21), 157.5 (NC); COSY, HMQC, and NOED spectra fully supported the proposed structure.

12-epi-*Hapalindole G* [**6**].—Uv (MeOH) λ max (ε) 222 (37220), 280 (8200), 291 sh (6640) nm; ir ν_{NH} 3395 cm^{-1}, ν_{NC} 2141 cm^{-1}; hreims *m/z* 338.1559 (calcd for $C_{21}H_{23}N_3{}^{35}Cl$, 338.1549); eims *m/z* 338/340 (100:34, M^+), 323/325 (37:11), 296/398 (25:8), 168 (40); ^{1}H-nmr data, see Table 1; NOED spectrum fully supported the proposed structure.

12-epi-*Hapalindole Q isonitrile* [**7**].—Uv (MeOH) λ max (ε) 222 (24160), 283 (4850), 291 sh (4300) nm; ir ν_{NH} 3419 cm^{-1}, ν_{NC} 2140 cm^{-1}; hreims *m/z* 304.1948 (calcd for $C_{21}H_{24}N_2$, 304.1939); eims *m/z* 304 (100, M^+), 221 (24), 168 (73), 130 (98); ^{1}H-nmr data, see Table 1; COSY and NOED spectra fully supported the proposed structure.

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